This approach will afford the Examiner the opportunity to reconsider the restriction requirement and one or more aspects of the nonelected subject matter on the merits.

As the current restriction requirement now stands, Applicants would need four to eight divisional applications (depending on rejoinder of the method claims) in order to cover all embodiments of the present invention, at great time and expense to file each application and then to issue plus maintain each resulting patent. The financial burden and reality of the situation will ultimately force Applicants to forego certain valuable claims. It is respectfully requested, therefore, that the new Examiner consider withdrawing the original requirement that restricted the invention to a single species and permit examination of the entire product claims or, at the very least, modify the restriction to comprise the *S. neurona* inactivated cells alone or in mixture with the *N. hughesi* inactivated cells, along with their respective antibody-inducing derived antigens.

To expedite matters in this case, nonelected Claims 23-25 drawn to a method for cell propagation are being canceled without prejudice to the right to file a divisional application directed to the nonelected subject matter of the invention.

The Examiner maintains the rejection of Claims 1, 2, 4-8 and 10-14 under 35 U.S.C. § 112, first paragraph, for reasons given on pages 3 and 4 of the Office action. In brief, the Examiner does not find that the specification is enabling for the use of any derived antigen to include single proteins that comprise epitopes for induction of a protective immune response. Applicants respectfully traverse the rejection for the following reasons.

Contrary to the Examiner's opinion, substantive evidence that the antigens of the present invention will be effective for their intended purpose of preventing infections is not required by statute. Working examples of each and every embodiment of an invention to prove absolute success are not necessary. In fact, there is no statutory prohibition against the possibility that the claims might include an inoperative antigenic substance. Routine screening is always permissible. Enablement only requires that the ordinary practitioner can practice the invention without an undue amount of experimentation.

In this case, the specification provides working examples that demonstrate that a vaccine of the present invention will be capable of inducing protective immunity in horses against equine protozoal myoencephalitis. It is well within the ordinary skill in the veterinary and pharmaceutical

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arts to be able to derive antigens from the whole cells of *S. neurona* and test their ability to prevent or ameliorate equine protozoal myoencephalitis infection or disease in similar studies.

The claimed subject matter is drawn to an antibody-inducing antigen derived from the whole cells of *S. neurona* (or *N. hughesi*). An acellular vaccine, which does not contain the whole cells, can be easily made from only the antigenic part of the disease-causing organism, for example, part of the protein cell wall. The specification teaches that the antigens are obtained using conventional procedures such as outer membrane extraction (see page 7, lines 8-11). The ordinary practitioner would appreciate how to derive the antigens from the whole cells by such standard and comparable techniques known in the art. Consequently, the enablement requirement is fully satisfied.

In view of the foregoing remarks, it is respectfully requested that the Examiner withdraw the rejection of Claims 1, 2, 4-8 and 10-14 under 35 U.S.C. § 112, first paragraph.

The Examiner believes that the Amendment After Final Rejection filed on May 12, 2005, inserted new matter into the specification by the addition of information with respect to the ATCC deposit made under the Budapest Treaty. Applicants are grateful that the Examiner kindly entered the Amendments to the Specification despite the holding of new matter. To address the Examiner's concern and complete the record, a true copy of the relevant ATCC Deposit Receipt is supplied herewith. The ATCC Receipt evidences that the deposit of the *Sarcocystis neurona* isolate designated SNg, having ATCC Accession No. PTA-2972, was duly deposited and accepted in the American Type Culture Collection under the provisions of the Budapest Treaty. Since this proof demonstrates that the added information is not new matter, Applicants respectfully ask the Examiner to withdraw her objection to the prior amendment.

The Examiner objects to Claims 1, 2 and 5 for certain informalities on pages 5 and 6 of the Office action. In response, the present amendment rewrites the claims for the better readability thereof and eliminates the semi-colons. Also, Applicants complied with the Examiner's suggestion to omit the comma between the phrase "merozoite antibody inducing" and "inactivated." To keep clear that these two phrases denote two different characteristics of the claim-recited *Sarcocystis neurona* cells, a hyphen has been added to the descriptive term "antibody-inducing." Should the Examiner have any further concerns regarding the claim language, she is invited to contact the below Attorney to discuss alternative options. Otherwise, it is

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respectfully requested that the objections to the claims be withdrawn.

The Examiner rejects Claims 1, 4-8 and 10-14 under 35 U.S.C. § 112, second paragraph, for grounds given in paragraphs 19-24 on pages 6-10 of the Office action. To advance the application towards an allowance, the present amendment revises the claim language for the better readability thereof, which obviates the Examiner's complaints. Since the amended claims use common, art-recognized terms, it is respectfully requested that the rejection be withdrawn.

Turning to the art rejections, the Examiner rejects Claims 1, 2 and 4-8 under 35 U.S.C. § 102(b) as allegedly being anticipated by Granstrom *et al.* evidenced by U.S. Patent No. 5,554,371 for reasons on pages 10 and 11 of the Office action. Applicants respectfully traverse the rejection.

Anticipation requires that there must be identity of invention. In this case, Granstrom *et al.* totally fail to anticipate the claimed invention because the reference does not describe the same entity as Applicants' immunogenically active component that is useful for preventing or ameliorating equine protozoal myoencephalitis ("EPM") infection or disease.

In order to make an inactivated or killed vaccine, it is critical that the *S. neurona* organism is unable to cause full-blown disease but still retains the antigens responsible for inducing the immune response in the horse. The specification illustrates a practical method of inactivating the whole cells of *S. neurona* wherein the merozoite harvests are inactivated by a formalin solution over a period of no less than 48 hours (see the top sentence on page 14 of the application). Granstrom *et al.*, on the other hand, does not teach or describe inactivated *S. neurona* cells or any functional antigen derived from *S. neurona* that could be injected into horses for the prevention or amelioration of EPM infection or disease.

The Granstrom *et al.* reference merely teaches antigens of cultured *S. neurona* merozoites for autopsy purposes. On page 90, col. 1, the authors indicate that their proteins are useful for antemortem detection of *S. neurona* exposure among clinical cases and potential candidates for development of an *S. neurona*-specific immunoassay. The authors are not proposing that their cultured *S. neurona* merozoites are effective to prevent or ameliorate EPM or safe enough to inject into horses. In practice, it is known from the literature that Dr. David Granstrom developed the first pre-mortem test for the presence of *S. neurona* antibodies in 1993, using a Western blot immunoassay. This diagnostic test is performed on serum and cerebrospinal fluid to detect the presence of antibodies to *S. neurona*, but the test is purely useful for diagnosis of antibodies. At

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the time the present application was filed, Dr. Granstrom and his colleagues had never taken their experiments further: They had neither developed nor described a vaccine or immunogenic composition that was useful to preventing the degenerative, protozoan disease of EPM.

One critical element of the claimed immunogenic composition that is clearly missing in the reference is the recited inactivated property of the *Sarcocystis neurona* cells to comprise an active component of a killed vaccine. The authors do not disclose any inactivated, immunogenic products that could induce an immune response in horses. Most importantly, Granstrom *et al.* never characterize their whole cells as being inactivated for vaccine use and do not describe the injection of inactivated merozoites into animals as an immunogen to prevent disease.

For preparation of sera or anti-sera in rabbits or horses, they inoculated the animals with live cultures of merozoites to achieve their stated objective in the abstract and title of examining antigens of **cultured** *S. neurona* merozoites using immunoblot analysis. The authors have no use for inactivated whole cells to reach their goal.

To prepare the immunoblots, the authors take purified merozoites, solubilize them with tris-Cl, 4% SDS (sodium dodecyl sulfate), 20% glycerol and 10% 2-mercaptoethanol, and then boil the mixture for one minute to finish the solubilization process before separating the solubilized merozoites by SDS-PAGE (sodium dodecyl sulfate - polyacrylamide gel electrophoresis). The purpose of SDS-PAGE is to separate proteins according to size. It does not determine any other physical feature or function of the proteins.

The anionic detergent SDS is added as a protein solubilization reagent. Heating in the presence of the detergent denatures the proteins of the solubilized merozoites. The liquid reagent 2-mercaptoethanol is used as an industrial solvent to dilute sera or other biological samples for electrophoresis studies. Glycerol is included as an aid to liquefy the sample. Tris buffer is added to control the pH in the presence of electrodes for electrophoresis.

Together with 2-mercaptoethanol, the standard 4% SDS mixture described by Granstrom *et al.* for resolving the sample by SDS-PAGE would not be used as a pharmacologically acceptable carrier for a veterinary vaccine formulation. Plus, the solubilized merozoites in the mixture of reagents are different from Applicants' inactivated whole cells and cannot perform the same functions.

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SDS denatures the proteins of Granstrom *et al.*, leaving them functionally altered from their native state. Denatured proteins lose most if not all of their biological activity. As a consequence of SDS denaturation, the structural change in the protein from the native state results in a molecule without secondary, tertiary or quaternary structure and with a large negative charge to help it migrate towards a positive pole when placed in an electric field. The solubilized and denatured protein of Granstrom *et al.*, being in an altered state, is not equivalent to an isolated, immunogenically active antigen that can find use in a vaccine to prevent or ameliorate EPM infection or disease as taught in the present application.

It is unclear how U.S. Patent 5,554,371 is being applied in the rejection. The '371 patent involves a recombinant vaccine against Lyme disease that comprises an antigenic recombinant polypeptide of about 110-kD molecular weight, as determined under reducing conditions by SDS-PAGE, wherein said polypeptide is combined with a physiologically acceptable, non-toxic liquid vehicle. The recombinant 110-kD polypeptide is obtained by the techniques of genetic engineering from a microorganism, such as a bacterial cell, which has been transformed using an appropriate vector with foreign DNA fragments obtained from the genome of a strain of *Borrelia burgdorferi*. A sole example of the non-toxic liquid vehicle carrier is described in the '371 patent as Freund's complete adjuvant.

To prepare rabbit and mouse antisera directed against the 110-kD *Borrelia* protein, the '371 patent discloses that the 110-kD protein band was excised from a Coomassie-blue stained SDS gel. The excised gel slice was frozen using liquid nitrogen and pulverized with a mortar and pestle. A small quantity of protein and acrylamide was injected subcutaneously. The rabbits or mice were reinjected with protein and blood was collected to prepare the antisera. It is plain to see that the '371 patent purely shows experimental use of the SDS gel to prepare antisera directed against the 110-kD *Borrelia* protein. SDS-PAGE gel is not a typical, pharmacologically acceptable carrier for an immunogenic vaccine or any veterinary composition. In fact, the '371 patent only demonstrates injecting the mice with the *Borrelia* protein in Freund's complete adjuvant for active and booster immunization, and certainly not the excised gel. Without any doubt, the art does not teach or imply that the SDS gel is an acceptable or conventional carrier for use in formulation of a vaccine.

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The Examiner also rejects Claims 1, 2 and 4-7 under 35 U.S.C. § 102(b) as allegedly being anticipated by Liang *et al.* (1998) for reasons given on pages 11 and 12 of the Office action. Applicants respectfully traverse the rejection.

The objective of the study in the Liang *et al.* article was to attempt to correlate immunoblot band patterns with *in vitro* neutralizing activity of equine serum and cerebrospinal fluid samples. From their limited comparative *in vitro* data, Liang *et al.* broadly suggest that two proteins, Sn14 and Sn16, might be surface proteins that might be useful components of a vaccine against *S. neurona* infection but, in reality, the authors purely invite the public to experiment further. The authors perform surface protein labeling, immunoprecipitation, Western blotting and typsin digestion to conclude that the two proteins appeared to be important for *in vitro* infection. To show sensitivity of *S. neurona* to specific antibodies in their *in vitro* serum neutralization test, Liang *et al.* had to artificially expose the *S. neurona* to antiserum for 10 minutes to yield a significant reduction in parasite production. They admit that Sn14 and Sn16 warrant further investigation because they find that their results may not be definitive.

Examining what the reference actually teaches, it is clear that Liang *et al.* do not describe the same immunogenically active component as Applicants claim in the present application because the reference does not show any isolated, immunogenically active antigen that would be useful for preventing EPM disease.

Liang *et al.* do not teach or disclose inactivating *S. neurona* whole cells for use in a killed vaccine. The authors merely dissolve the cells in standard SDS-PAGE buffer and heat the mixture sample in boiling water for five minutes to separate in an SDS linear gradient polyacrylamide gel for immunoblot analysis. It is clear that the authors were not trying to inactivate the whole cells; and they were not using the PAGE gel as a carrier to make a vaccine product. Rather, they were using the art-recognized technique of solubilizing and denaturing the protein of the *S. neurona* cells to analyze the protein molecular size by resolving the proteins in SDS-PAGE. Liang *et al.* simply use the heated mixture sample for immunoblotting or immunoprecipitation procedures but never describe obtaining inactivated whole cells, harvesting the cells or injecting the cells for use as an immunogenically active component to prevent or ameliorate EPM infection or disease as claimed herein.

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Moreover, Liang *et al.* only compared *in vitro* neutralization of *S. neurona* infectivity by serum and CSF samples but they never isolate any protein, never prepare a composition of any protein and never inject the protein into horses. The correlation of band patterns with inhibitory activities that suggests that two proteins, Sn14 and Sn16, may be surface proteins that appear to be important for *in vitro* infection truly does not describe or anticipate an antibody-inducing antigen derived from the whole cells of *S. neurona*. There is no question that the reference does not anticipate the claimed invention.

The Examiner further rejects Claims 1, 2, 5 and 8 under 35 U.S.C. § 102(e) as allegedly being anticipated by Mansfield *et al.* (U.S. Patent No. 6,489,148) for reasons explained on pages 12 and 13 of the Office action. Applicants respectfully traverse the rejection.

The '148 patent of Mansfield *et al.* relates to an immunoassay for the detection of disease caused by *S. neurona* in equines which consists of: (a) isolating fluid from the equine which can contain parasite induced antibodies to *S. neurona* proteins, indicating the presence of the *S. neurona*; (b) reacting the fluid with at least one identifying antigen of the *S. neurona* protein bound on a substrate, wherein the substrate has been blocked with antibodies to *Sarcocystis* sp. other than *S. neurona* so that antibodies to *S. neurona* antigen in the serum are bound to the identifying antigen; and (c) detecting the antibodies bound to the antigen so that the disease is detected. Mansfield *et al.* claim that their positive criteria and blocking technique improve the specificity of the conventional Western blot antibody test for *S. neurona*.

To perform their Western blot test to detect antibodies to *S. neurona*-specific antigens in cerebrospinal fluid of horses suspected of having EPM, Mansfield *et al.* describe harvesting *S. neurona* merozoites from equine dermal cell culture, denaturing the cells by heating in sample buffer and separating the denatured proteins by SDS-PAGE in 12-20% linear gradient gels with a 4% stacking gel. Separated proteins are electrophoretically transferred to Westran PVDF membranes and blocked overnight in blocking buffer. The denatured and separated proteins are never admixed with a veterinary carrier for inoculation of horses. They are employed solely as a diagnostic tool.

Moreover, the heat-denatured cells are not equivalent to the immunogenic composition or vaccine of the present invention. Because the denatured proteins have been physically altered from their native state, there is no biological equivalence to the claim-recited antigen that can

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induce an immune response in horses to prevent or ameliorate infection or disease. Mansfield *et al.* do not describe inactivated whole cells of *S. neurona* in any immunogenic composition or vaccine useful for preventing or ameliorating EPM infection or disease. Thus, anticipation cannot be seen.

Lastly, the Examiner rejects Claims 1, 2, 4-8 and 10-14 under 35 U.S.C. § 102(b) as allegedly being anticipated by Dubey *et al.* (J. Eukaryot. Microbiol., 1999) for reasons set forth on pages 13 and 14 of the Office action. Applicants respectfully traverse the rejection.

Dubey *et al.* fail to describe the claimed invention. The authors disclose the use of buffered neutral formalin for fixing tissue for histopathologic and immunohistochemical examinations but they do not use formalin to inactivate whole cells of *S. neurona*. They do not describe inactivating the merozoites by a formalin solution over any length of time in order to make a killed vaccine, let alone an effective time of no less than 48 hours as described in the present application.

To prepare anti-*S. neurona* polyclonal sera in rabbits, Dubey *et al.* describe immunizing the rabbit with a recent isolate of *S. neurona* from the feces of a naturally-infected opossum through three inoculations. For the first and second immunizations, live, filtered organisms from an equine dermal cell culture were injected into the rabbits. For the third immunization, filtered organisms from a bovine turbinate cell culture were frozen, thawed and mixed with immunostimulating adjuvants. In this regard, there is no indication that the third inoculation contained inactivated *S. neurona* cells. Whole cells can certainly be frozen without inactivating the protozoa. Plus, the authors' goal was to produce anti-*S. neurona* polyclonal sera, and not immunize the rabbits from EPM infection or disease. Live culture would be better suited for their goals.

Dubey *et al.* even admit on page 504 that little is known of the development and antigenic composition of *S. neurona* merozoites. The authors further state that unless the culture conditions are standardized, variability in antigenic composition of cultured *S. neurona* cannot be controlled. Dubey *et al.* do not propose or describe the use of an inactivated culture of *S. neurona cells* as an immunogenic composition or vaccine.

Considering the art, none of the references cited by the Examiner, namely Granstrom *et al.*, Liang *et al.*, Mansfield *et al.* and Dubey *et al.*, anticipate the present invention. The

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references fail to describe Applicants' immunogenically active components that can prevent or ameliorate equine protozoal myoencephalitis infection or disease. Indeed, the references lack any teaching of immunogenic compositions or vaccines capable of inducing protective immunity against EPM.

As the Examiner has found, the art is full of many articles dealing with the diagnosis of EPM that is a highly prevalent and devastating disease in horses. The etiology appears to be clear but protection of the horses from getting the infection or disease is not. There is an overwhelming demand in the horse industry to find a vaccine that prevents or ameliorates this debilitating, neurological disease of horses. Applicants' introduction by Fort Dodge Animal Health (a division of Wyeth) of the first *S. neurona* vaccine on the market is substantiating proof that no one has heretofore described a vaccine that protects against EPM infection or disease. Anticipation based on the art simply cannot be sustained.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejections of the pending claims based on the cited references and allow the present application.

Accordingly, favorable treatment is respectfully urged.

Respectfully submitted,

WYETH

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